# ON THE PRESENCE OF *LEISHMANIA* IN THE DIGESTIVE TRACT OF *ANOPHELES MACULIPENNIS*

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PLATES II, III, IV

In a previous paper, published in the 'Lancet' (1911, *a*) and 'Pathologica' (1911, *b*), I have described the different methods employed in my researches on *Anopheles maculipennis*. I have in this paper given the reasons on which my statement was based that the Leishman bodies could live for a certain length of time, and in some cases multiply, in the digestive tract of the above mosquito. I also stated that I believed the results to be of great interest, as I had never found the Leishman bodies in smears (fixed in methyl-alcohol and stained with Giemsa) from the intestines of *Cimex lectularius* and *Pulex serraticeps* and *P. irritans*, which had, in my presence, fed on artificial cultures containing numerous parasites.

As regards *Pediculus capitis*, the conditions of experiment were rather unfortunate owing to the difficulty experienced in inducing the insects to feed on the cultures, and owing to the short time the insects live outside their natural habitat. I did not find any Leishman bodies in the smears made from a few of these insects (*P. capitis*), although they appeared to me to have taken up some of the cultures.

My experiments comprised several hundred, with numerous controls (i.e., with insects which had not been fed on cultures), in order to see what other parasites normally inhabit the intestine of *A. maculipennis*, knowing that flagellates had previously been found in the digestive tract of mosquitos.

As regards *A. maculipennis*, I have examined more than 300

smears, and I have convinced myself that the parasites (Leishmania) found by me could not have been mistaken for other similar parasites, as they can be distinguished by their nucleus, different staining, and generally much larger size. Besides, these flagellates, as far as I was able to ascertain, are rather scarce.

The reasons which lead me to believe that the Leishman bodies can live in the digestive tract of *A. maculipennis* are as follows:—

1. The conspicuous staining of the different parts of most of the parasites.

2. The frequent presence of large numbers of parasites.

3. The fact that, if the parasites had not been alive, they would in a short time have been destroyed by the digestive juices as well as by the numerous cocci, bacteria and other parasites found in the intestine.

Objection has been raised to my work on this subject, that the parasites found by me were well-stained degeneration forms. To say the least, it would be strange that degeneration forms should stain as well as the living organisms. A comparison between the parasites, illustrated in Plates II, III, IV, and drawn from stained preparations, and those of living cultures containing numerous parasites, will show no impaired development of the former. I do not deny that there may perhaps be degeneration forms amongst the bodies found by me—but does this never occur in cultures? Further, objection has also been made to my statement that the parasites were living in the digestive tract, that the mosquitos had, together with the parasites, also taken up some of the culture medium. It may be possible that the culture medium does assist the development of the parasites in the digestive tract of *A. maculipennis*, although I have not found this to be the case in *P. serraticeps* and *P. irritans*, which likewise had taken up the culture medium together with the parasites.

Nor is it possible that the Leishmania could live for a greater length of time in a culture medium outside the test-tube, or exposed to the action of bacteria. It has, therefore, to be admitted that there exist in the digestive tract of *Anopheles maculipennis* and other Culicidae including *Stegomyia fasciata*, special conditions favourable to the life of Leishmania, and that these conditions do not exist in the other insects experimented upon.

In all the experiments on the different insects, I have used well-developed cultures containing numerous parasites. It seemed to me that the number of the parasites found in the digestive tract of the mosquitos was greatest, and the forms most varied in cases where the insects were still partially filled with blood from a previous meal on man or on animals. In some preparations nuclear changes (spindles) were observed. Is it not reasonable to suppose that that which occurs when mosquitos have fed on cultures, will also happen when they feed on men or animals? If we suppose that the Anopheles is the transmitting agent of the disease, would it not also ingest the blood in which the parasites are contained? It is well known that these mosquitos are very bloodthirsty. As it has been suggested that the dog might be the intermediate host (though I doubt the correctness of this hypothesis since the percentage of infected dogs is too high in localities where Kala-Azar is extremely rare, at least in Italy), the mosquito would ingest the parasites together with blood, and the same would be the case in the transmission from man to man during the period in which the parasite is present in the circulation. The Leishmania thrive on nutritive media containing blood, and one may infer that the blood is a necessity for their life and development.

I believe I am justified in saying, now, after more numerous experiments, that the parasites not only live but also develop, which is evident from the figures in the Plates.

Plate III shows clusters of parasites and rosette forms; it has hitherto been doubted that development took place because these forms did not occur. According to my experience the groups and rosettes demonstrate very little, owing to the great tendency of the Leishmania to form clusters and adopt most varied shapes. But the important points of my researches are to have found:—

1. Among the parasites derived from the intestine of mosquitos very numerous forms of division, into two or three, as generally found in well-developed cultures (see Plates II and III).

2. Modifications in the shape and structure of the parasites derived from the intestine of mosquitos as compared with those obtained from the culture on which the insects had been fed.

3. Karyokinesis of the nucleus occurring in some of my preparations.

I have already stated in another paper (1911, *b*) that I found in preparations from the intestines of mosquitos, fed on cultures of from ten to fifteen days, small round forms with nucleus and blepharoplast identical with those contained in the internal organs of individuals infected with Kala-Azar. These forms can also be found in cultures, but only after some considerable length of time.

In more recent experiments, I have employed, instead of flagellate forms, the round, non-flagellate forms found in culture tubes after some time had elapsed. Attention must be paid to the fact that these forms were not only capable of life, but also of multiplication, for flagellate forms developed in culture tubes from the non-flagellate forms used for experiment.

In this way I obviated the objection made that the flagellate forms found by me were not Leishmania. I obtained also from the intestine of the mosquitos numerous parasites which were well preserved, partly flagellated and, in some instances, so numerous as to resemble actual cultures. Therefore, the non-flagellate forms can live in the intestine of *A. maculipennis* as well as the flagellate forms.

Besides the modifications in the shape of the parasite and nucleus, there appeared to me to be also modifications in the structure of the protoplasm which stained more intensely, and at times appeared granular, as I found it only in the parasites from the intestine of the mosquitos.

As regards the presence of granules in the parasite, this is a point which merits still further researches, considering the immense numbers of cocci present in the digestive tract of mosquitos as seen in Plate II. It might be that it is simply a case of superposition of the cocci over the parasites, although the number of the granules within the periphery of the parasite is often much greater than that of the cocci in the field outside the parasite. The examination of fresh preparations does not help very much in answering the question.

In the faeces of mosquitos excreted at various times after the insect had been fed on the cultures, fair numbers of parasites of various forms were found, partly isolated and partly in groups. They were, to a great extent, well preserved, as seen in Plate IV.

This shows to perfection that the Leishmania can pass the digestive tract of *A. maculipennis* without being destroyed, and sometimes not even altered. Therefore, more or less the same occurs here, as in the case of *A. maculipennis* and the malarial plasmodium. In fact, true excretions of malarial parasites have been described from infected mosquitos.

I had wished to obtain cultures from the Leishmania found in the digestive tract of *A. maculipennis*, more especially as objections have been raised that I had not made these cultures, an experiment which is certainly most difficult.

It is not always possible to obtain cultures from the splenic juice in which there are numerous Leishman bodies, and other blood parasites or bacteria. In the digestive tract of *A. maculipennis*, however, there are very numerous cocci, which in Novy-MacNeal-Nicolle's medium develop so rapidly during the first twelve hours that they impede the life and development of the Leishmania. It must also be remembered that the Leishmania obtained from the splenic juice and inoculated on the above medium require from at least three to four days for development, and that the parasite does not grow on a non-sterile medium, and dies as soon as other bacterial growth sets in.

There is, consequently, all the more reason to believe that, if the Leishmania can live in the digestive tract of *A. maculipennis*, there must exist, in the intestine, exceedingly favourable conditions which do more than compensate the possible destructive action of other germs present, or of the digestive juices themselves. The virulence and behaviour of the numerous cocci, in the intestine of the insect and in cultures, is certainly not the same; and I, therefore, did not waste any time in attempting to make cultures, but I fixed the mosquitos and embedded them in paraffin in order to cut sections, and examine especially the salivary glands. I have already made numerous sections, stained with haematoxylin and eosin, and the results seem to be encouraging, but, owing to the importance of the argument, I do not wish to draw hasty conclusions before more numerous experiments have been made.

The experiments had to be discontinued at the beginning of November, when, owing to the fall of temperature, no insects could

be obtained. They will be resumed on a large scale next spring, especially the animal experiments. It is necessary to transmit the infection from the mosquito to animals susceptible to infection with the parasite of Kala-Azar, such as monkeys, dogs, etc.

The experiments considered in this paper are, as I stated previously, only the beginning of a series of researches up till now made in the laboratory, and I have never attached more importance to them than merited by the results obtained.

I do not wish to discuss the question of the identity of the Kala-Azar of India and that of the Mediterranean, as this does not come within the range of my argument. I am of opinion that, in Italy, the possibility of a transmission of the Leishman parasites by means of the Anopheles does not meet with any difficulty. In many parts of Italy, where Kala-Azar is present as well as malaria, cases of both diseases have occurred to my knowledge in one and the same family. There are other countries round the Mediterranean where malaria and Kala-Azar co-exist. Nicolle has seen many cases of Kala-Azar in Tunis and suggests experiments on the mosquito. A study of the distribution of the Anopheles mosquito in countries infected with Kala-Azar is not only useful but also necessary.

It has been stated that the yearly recurrence of Kala-Azar in spring, which has been observed in some parts of Italy, is contradictory to the transmission of the Leishmania by means of the Anopheles mosquito.

I am, however, of opinion that the recurrence of the disease at a certain time of the year cannot be a valid objection considering our complete ignorance about the incubation period (I may also mention that it is difficult to recognise the onset of the disease). I would like to mention that the period of incubation varies greatly in different animals, dogs, monkeys, etc. We cannot, therefore, draw a parallel between the number of cases of Kala-Azar and the number of bites of the Anopheles mosquito. It has been stated that cases of Kala-Azar occurred with greatest frequency in spring, and that the Anopheles bites most frequently in July, August, and September, and this may very well be so. Might it, however, not also be possible that the incubation period in man was as long as the time elapsing between these months and spring? If we admit the

transmission of Kala-Azar by the Anopheles mosquito, it seems to me most probable that such would explain the occurrence of cases in countries far away from primary foci.*

I have made experiments on other mosquitos, and found that the Leishmania can also live in the digestive tract of some Culicidae, including *S. fasciata*, although not as frequently and abundantly as in *A. maculipennis*.

## CONCLUSIONS

After numerous experiments on *Pulex irritans* and *P. serraticeps*, as well as *Pediculus capitis* and *Cimex lectularius*, which had been fed on cultures of Leishmania, no parasites were found which could be identified with Leishman bodies, and I am of opinion, therefore, that further observations should be made with mosquitos, especially *Anopheles maculipennis* where this mosquito exists.

The Leishman bodies are present in the digestive tract of *A. maculipennis* in great numbers and in various stages of development, as seen in Plates II-IV.

The staining of the different parts of the parasites, viz., nucleus, protoplasm and blepharoplast, is so evident that it must be admitted that the parasite is alive and capable of reproduction.

In some of the parasites true nuclear karyokinesis has been observed.

Wenyon (1911) of the London School of Tropical Medicine, in an exhaustive and interesting monograph on the Oriental Sore of Bagdad, has found that *Leishmania tropica* can live and develop in the intestine of *Stegomyia fasciata*. From the non-flagellate form of Oriental Sore he obtained the flagellate form in the intestine of *S. fasciata*, that is to say, the same occurred in cultures of *Leishmania tropica* as in the case of *L. infantum*.

## REFERENCES

Franchini, G. (1911, *a*) 'Note on Leishmania and Mosquitoes,' The Lancet, November 4, pp. 1268-1269.

Franchini, G. (1911, *b*) 'La Leishmania pui vivre e svilupparsi vel tubo digerente dell *Anopheles naculipennis*.' Pathologica, November 1, No. 72, pp. 611-613.

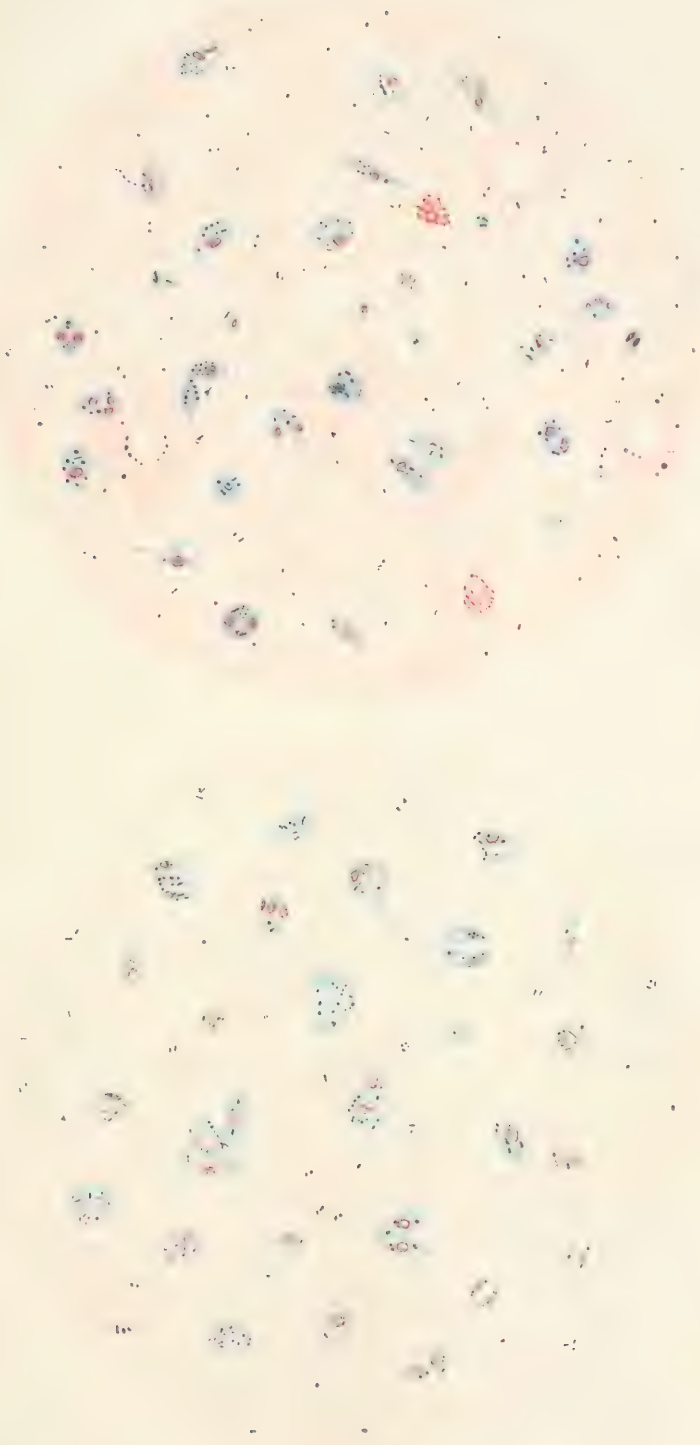
* In Italy we find examples of cases of Kala-Azar which are far apart and there has been absolutely no contact with infected patients. Infection by bugs and fleas could be definitely excluded because there was no contact with either the actual patient or with the clothes of an infected patient, and in some cases there were no animals in the houses, so we can therefore exclude the possibility of these acting as carriers of the disease in these cases.

## EXPLANATION OF PLATE II

The figures have been drawn with Zeiss, Obj. 6, Comp. oc. 8, and show two microscopic fields with numerous Leishmania derived from the digestive tract of *Anopheles maculipennis*, fixed in absolute methyl-alcohol, and stained with Giemsa.

Fig. 1 shows, besides the Leishman bodies, numerous bodies and other parasites which stain red with Giemsa. The other parasites were also met with in fleas and bugs. The Leishmania were very numerous and present all the different stages of development, from the small, non-flagellate forms found in the haematopoietic organs of patients with Kala-Azar to the flagellate forms of cultures. It will be noted that numerous dividing forms are present.

Fig. 2 shows numerous Leishman bodies, nearly all non-flagellate, from the more or less rounded to the oval forms. The smears containing these parasites were obtained from mosquitos which had lived for a much longer period than those from whom the parasites in fig. 1 were obtained. The size of the parasites varies exceedingly; forms are present which show more than one nucleus and some of which are filled with chromatin (?) granules. Some forms, which are rare, appear about to degenerate.



## EXPLANATION OF PLATE III

Figures 1-19 have been drawn with Zeiss. Obj. 8, Comp. oc. 8; and Figures 20-35 with Zeiss. Obj. 6, Comp. oc. 8.

Fig. 1. Rosette form with interesting modifications of the nuclei which are better seen with a higher magnification. Close by two small non-flagellate parasites with nuclei and blepharoplast as in the parasites found in the spleen.

Figs. 2-5. Parasites about to divide; figs. 3-5 with two nuclei and two blepharoplasts.

Fig. 6. Common flagellate form.

Fig. 7. Globular form with central nucleus. The nucleus contains little masses of chromatin.

Fig. 8. Large form showing cystic appearance, with numerous nuclei and blepharoplasts.

Fig. 9. Form with two nuclei and numerous blepharoplasts.

Fig. 10. Groups of parasites, some of which are non-flagellate.

Fig. 11. Globular parasite with three nuclei and one blepharoplast.

Fig. 12. Groups of parasites, some flagellate, others non-flagellate.

Figs. 13-14. Dividing forms.

Fig. 15. Dividing form (?).

Fig. 16. Dividing form with polar grouping of chromatin; at a higher magnification chromatin filaments can be seen.

Fig. 17. Group of well stained parasites.

Figs. 18-19. Some modifications of the nuclei as in fig. 16.

Figs. 20-24 and 26. Dividing forms with enormous numbers of chromatin granules in the protoplasm.

Fig. 27. Group of parasites of various forms.

Fig. 29-30. Small oval forms with flagellum just indicated and with protoplasm stained intensely blue. They are identical with the first stages of development found in cultures, neither nucleus nor protoplasm differentiated.

Fig. 31-32. Globular forms with many nuclei.

Fig. 33. Apparently a cystic form.

Fig. 34. As figs. 31, 32.

Fig. 35. Globular form.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## EXPLANATION OF PLATE IV

Figs. 1-24 show parasites derived from the digestive tract of some culicides and from *Stegomyia fasciata.*

Figs. 2, 7, 8, 9, 12, 14, 16, 17, 21, 23 represent dividing forms.

Fig. 18. Parasites with their protoplasm partly rose and partly an intense blue, similar to some forms of the first stages of development.

Figs. 19, 20, 22. Groups of parasites of various forms, some with one blepharoplast and two nuclei.

Fig. 24. Probably a joined form of three parasites with short flagella.

The second part of the figure shows figures of Leishmania which have been derived from the faeces of Anopheles, which had fed on cultures. In many of the parasites the protoplasm, nucleus and blepharoplast are intensely stained.

Figs. 1-4 and 14. Parasites similar to those found in the internal organs of Kala-Azar patients.

Fig. 7. Group of four parasites, in one of which only is the flagellum preserved.

Fig. 16. Probably a dividing form.

Many of the other parasites have lost their flagellum and show two nuclei.


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